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Wendan decoction improves learning and memory deficits in a rat model of schizophrenia☆

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Abstract

An experimental model of schizophrenia was established using dizocilpine (MK-801). Rats were intragastrically administered with *Wendan* decoction or clozapine for 21 days prior to establishing the model. The results revealed that the latency of schizophrenia model rats to escape from the hidden platform in the Morris water maze was significantly shortened after administration of *Wendan* decoction or clozapine. In addition, the treated rats crossed the platform significantly more times than the untreated model rats. Moreover, the rate of successful long-term potentiation induction in the *Wendan* decoction group and clozapine group were also obviously increased compared with the model group, and the population spike peak latency was significantly shortened. These experimental findings suggest that *Wendan* decoction can improve the learning and memory ability of schizophrenic rats to the same extent as clozapine treatment.

Key Words

Wendan decoction; schizophrenia; Morris water maze; long-term potentiation; hippocampus; learning and memory; traditional Chinese medicine; neural regeneration

Abbreviations

WDD, *Wendan* decoction; LTP, long-term potentiation; PS, population spike

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INTRODUCTION

Wendan decoction (WDD) is widely used in clinical applications in Chinese medicine, especially in depressive and manic psychosis, which is similar to schizophrenia[1-8]. To treat schizophrenia in traditional Chinese medicine, physicians typically start by examining phlegm and focusing on regulating *qi* to resolve the phlegm. WDD is a traditional method for treating psychiatric diseases induced by invisible phlegm. Schizophrenia is associated with a range of cognitive impairments, affecting memory, attention, abstract thinking and information integration[9].

In current models of schizophrenic rats, it is widely accepted that hyperlocomotion and stereotyped action are commonly manifested psychiatric symptoms in model rats, in addition to dysfunctional cognition[10]. The animal model of schizophrenia induced by dizocilpine (MK-801) is widely considered one of the best experimental methods[10]. MK-801 affects short-time memory, memory retention and consolidation, and the formation of long-term memory in model rats[11]. It is currently unclear whether WDD improves learning and memory deficits in the rat model of schizophrenia. As such, the present study sought to investigate the

influence of WDD on learning and memory through behavioral examination of the Morris water maze task, and electrophysiological analysis of the effects of WDD on learning and memory.

RESULTS

Quantitative analysis of experimental animals

A total of 80 rats were randomly and equally divided into four groups, namely normal (normal rats), model (schizophrenic rats), WDD (schizophrenia + WDD) and clozapine (schizophrenia + clozapine). Ten rats in each group were tested in a Morris water maze experiment, and the other 10 were tested in a long-term potentiation (LTP) experiment[12].

WDD increased the success rate of LTP induction in hippocampal neurons of schizophrenic rats

The electrophysiological results revealed that the reaction of population spike (PS) in area CA1 of the hippocampus have been recorded in 8 of 10 rats in the WDD group and clozapine group, 5 of 10 in the normal group and 3 of 10 in the model group. Using the H-rank test, the success rate of LTP induction in the model group was significantly lower than in the normal, WDD and clozapine groups ($P < 0.05$ or $P < 0.01$), indicating that PS in the hippocampus of schizophrenia model rats was markedly inhibited. However, WDD treatment was found to cause a clear decrease in the inhibition of PS in the hippocampus of schizophrenia model rats (Table 1).

Table 1 The success rate of long-term potentiation induction in each group

Group	Number of provocation	The rate of provocation (%)
Normal	5	50[a]
Model	3	30
Wendan decoction	8	80[b]
Clozapine	8	80[b]

There are 10 rats in each group. [a]$P < 0.05$, [b]$P < 0.01$, *vs*. model group (H-rank test).

WDD shortened PS latency in hippocampal neurons of schizophrenia model rats

Compared with the model and normal groups, PS amplitude in the WDD and clozapine groups was increased, and PS latency was shortened after high frequency stimulation, typically by around 1–2 ms. However, the normal and model groups did not exhibit this trend (Figure 1).

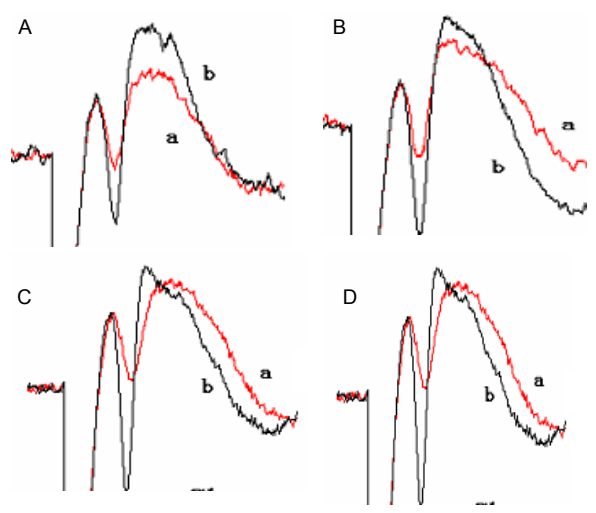

Figure 1 Comparison of population spikes in the dentate gyrus of the hippocampus of rats in each group.

a (red): Before high frequency stimulation; b (black): after high frequency stimulation.

After high frequency stimulation, population spikes in the *Wendan* decoction group (C) and clozapine group (D) were higher than those of model group (B) and normal group (A), and population spikes in C and D were approximately 1–2 ms shorter.

WDD shortened the escape latency of schizophrenia model rats in the Morris water maze

On days 1 and 2 of spatial discrimination training, the latency in each group was not significantly different ($P > 0.05$). However, from the third day, the escape latency in the model group was longer than in the normal, WDD and clozapine groups ($P < 0.05$ or $P < 0.01$). The escape latency of rats in each group decreased with training days (Table 2).

Table 2 Influence of *Wendan* decoction on the average escape latency (second) in schizophrenia model rats

Group	Training time (day)		
	1	2	3
Normal	48.18±13.53	44.35±14.52	40.31±12.43[a]
Model	50.88±14.12	49.78±9.68	49.85±11.77
Wendan decoction	47.68±13.48	43.59±3.29	42.69±12.97[a]
Clozapine	47.12±11.58	43.05±5.38	41.18±14.36[a]

Group	Training time (day)	
	4	5
Normal	36.06±9.07[b]	27.53±18.87[b]
Model	48.31±6.45	46.43±13.89
Wendan decoction	39.44±10.27[b]	33.81±15.02[b]
Clozapine	37.32±12.26[b]	30.21±16.55[b]

Data are expressed as mean ± SD, there were 10 rats in each group. [a]$P < 0.05$, [b]$P < 0.01$, *vs*. model group (one-way analysis of variance).

In addition, the escape latency of normal, WDD and clozapine groups was significantly shorter at day 2 compared to day 1. Escape latency then markedly declined over later days. However, the escape latency of the model group was not substantially shortened.

WDD increased the number of times the platform was crossed in schizophrenia model rats

The spatial exploration test showed that rats in the normal group exhibited a high level of searching near the location of platform, crossing the platform location many times. The number of times the platform was crossed in the model group (0.91 ± 1.12) was significantly less than in the normal group (3.02 ± 1.58), WDD group (2.41 ± 1.65) and clozapine group (2.69 ± 1.33).

The relative amount of activity spent in the outer ring region in the WDD and clozapine groups was significantly less than that in the model group ($P < 0.05$). However, the relative amount of activity spent in the central zone in the WDD and clozapine groups was significantly increased ($P < 0.05$; Table 3).

Table 3 The percentage of activity in the outer ring, the central ring and the central zone

Group	The outer ring	The central ring	The central zone
Normal	66.21±18.45[a]	30.50±18.42[a]	2.79±2.46[a]
Model	83.37±13.72	14.87±13.21	1.39±2.28
Wendan decoction	62.30±16.12[a]	33.49±15.36[a]	4.33±1.79[a]
Clozapine	64.69±10.03[a]	32.15±13.47[a]	3.94±1.51[a]

Data are expressed as mean ± SD, there are 10 rats in each group. [a]$P < 0.05$, *vs.* model group (one-way analysis of variance). The outer ring is approximately 20% of the water maze ring, while 40% is the central ring, and the rest is the central zone (supplementary Figure 1 online).

DISCUSSION

Studies of the psychological, neurophysiological and biochemical mechanisms of memory indicate that the memory process includes acquisition, retention and recall. The links between these three stages are different but interact with one another[5]. In the present study, the learning and memory ability of schizophrenic model rats established with MK-801 were tested in the Morris water maze. The behavioral results indicated that learning and memory were substantially impaired in schizophrenia model rats, but that WDD treatment improved it to some extent. The results of the spatial exploration test revealed that the model group (untreated) searched longer near the location of platform than animals in the other groups (treated). The number of times the platform was crossed was markedly reduced in the model group (untreated) compared with the other groups (treated). This finding indicated that the model rats exhibited spatial learning and memory deficits, which WDD treatment was found to improve.

Rats exhibited an instinctive searching routine around the tank wall in the first day because they did not know the location of the platform. Most rats exhibited similar search strategies, and a few rats swam to the center of the tank. On the second experimental day, rats in the normal, WDD and clozapine groups exhibited a tendency form while those in model group still exhibited a stochastic pattern of behavior. After five days of training and learning, learning and memory ability in model group rats was significantly lower than in the other groups. The model group tended to exhibit a stochastic search pattern after entering water, while the other groups tended to move in a straight line pattern. These results suggest that schizophrenia model rats exhibited learning and memory deficits that could be ameliorated by both WDD treatment and clozapine treatment, to the same extent. These results are consistent with the findings of recent research from our laboratory[12].

In addition, in the LTP induction test, after high frequency stimulus activated the perforant path-dentate gyrus pathway, rats in the WDD and clozapine groups exhibited higher rates of successful induction of PS compared with the untreated model group. In addition, the PS amplitude increased in the treatment groups compared to the model group, and this increase lasted more than 60 minutes. PS in the dentate gyrus of granulocytes substantially declined in the hippocampus of model rats. WDD administration led to obvious improvements, boosting the transmission of glutamic acid synapses in area CA1 of the hippocampus within a short time. In addition, the PS amplitude in each group was increased after receiving high frequency stimulation, and the rats in WDD group exhibited a shortened peak latency. These results suggest that by affecting the transmission level of cerebral amino acids, WDD enhanced the binding of neurotransmitters and their receptors, boosting the free calcium concentration of the postsynaptic membrane. This would be expected to facilitate synaptic conduction, affecting formation of hippocampal neuronal plasticity and promoting information acquisition and memory consolidation. A better understanding of the mechanisms underlying this process will require further study.

MATERIALS AND METHODS

Design

A randomized, controlled animal experiment.

Time and setting

The experiment was conducted in the National Key

Laboratory of Ministry of Education, Jiangxi University of Traditional Chinese Medicine, China, during 2009 and 2011.

Materials

Experimental animals

Eighty 1.5-month-old female Sprague-Dawley rats, specific pathogen free grade and weighing 200 ± 20 g, were supplied by Experimental Animal Center of Jiangxi University of Traditional Chinese Medicine (license No. 2005-0001). The experiments were approved by the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China[13].

WDD preparation

Pinellia tuber 10 g, pinellia tuber 30 g, bamboo shavings 10 g, young orange fruit 10 g, dried tangerine peel 10 g, and radix glycyrrhizae 6 g (a total crude drug amount of 76 g per dose) were all provided by Jiangxi Provincial Hospital of Traditional Chinese Medicine, China. All of the drugs were immersed in water for 30 minutes. First, the crude drugs and eight times their volume of water were put in a container to decoct 40 minutes after boiling. The liquor was then preserved after being filtered. Second, six times more water was added into the container to decoct 30 minutes after boiling, and the liquor was preserved after being filtered. The preserved liquor was put together and concentrated until the concentration ratio of drug to water reached 1:1, the decoction (1 mg/mL) was preserved in the refrigerator for use under 4°C[14].

Preparation of clozapine liquor

Ten clozapine tablets (5 mg/tablet, production code: 0812161; Jiangsu Ruinian Progressing Limited Company, Yingxing, China) were dissolved in 50 mL sodium chloride after the tablets were ground. The clozapine liquor was prepared upon use[15-16].

Methods

Drug administration

Rats in the WDD group and clozapine group were intragastrically administered with 20 mL WDD, or 20 mL clozapine liquor, respectively, per kg body weight. This is equal to 20 mg/kg for adults[17-18]. The normal and model groups were administered the same volume of sodium chloride. The rats in the four groups were given drugs once a day from 8:00 a.m. to 9:00 a.m. for 21 days.

Model establishment

The experiments began shortly after the rats had adapted to the rearing environment for 5 days. Except for the normal group, the rats in other three groups were injected with MK-801 (Sigma, St. Louis, MO, USA) within 1 hour after administration at the left abdominal cavity under the dosage of 0.6 mg/kg to establish rat models of schizophrenia[19-21] after being administered drugs for 21 days. The rats in normal group were injected with sodium chloride (20 mL/kg). The Morris water maze and LTP experiments were conducted 3 days after establishing the models.

Morris water maze for assessment of learning and memory ability

Spatial discrimination test: Vistascope (Beijing Public Shidi Technology Development Limited Liability Company, Beijing, China), installed above the water tank, was connected to a computer. The camera driving procedure and the picture collection processing system (Beijing Public Shidi Technology Development Limited Liability Company) were installed after starting the computer. On day 1, all rats were put into the water tank and allowed to swim freely without a platform in the tank for 1 minute to familiarize and adapt to the Morris water maze. Pool specifications: diameter 150 cm, high 60 cm (Beijing Public Shidi Technology Development Ltd.). At the same time, the swimming pose and speed were examined. Rats whose swimming posture and speed differed from that of normal rats were not included in the experiment. On day 2, the safety platform was placed into the south-west quadrant of the water tank, and training began. Different quadrants were selected when the rats entered the water, and they were placed so that they faced the tank wall when entering the water. The time from the rat entering the water to touching the platform (escape latency period) and the search strategy were examined[22]. If the rats could not find the platform within 60 seconds, the test stopped.

Rats were trained twice a day: two times in the morning and two times in the afternoon. The rats rested for 30 seconds after they climbed onto the platform, then began the next test. Testing was conducted for 5 days. Two scores in every training session were selected to calculate the arithmetic mean. We considered the arithmetic mean as an index of performance in each training session.

Spatial exploration test: After 5 days of spatial discrimination, each rat was tested in the water tank with the safety platform removed. Rats usually searched for the platform, and swam around the platform's previous location. We recorded the total number of times the previous platform location was crossed. This number was considered to represent rats' memory ability. In addition, we recorded a line map of activity across the platform for the 60-second period.

LTP experiment

An extracellular recording method was used to observe and record the induced potential. The stimulation line was connected to a stimulating electrode (Beijing Public Shidi Technology Development Limited Liability Company), the recording line was connected to the proceeding cable, and the stimulating electrode was placed in the entorhinal area before the perforant path. The hippocampal electrode was placed in the dentate gyrus granular cell layer. The stimulating line and stimulating electrodes were connected together. The zero line was connected to the rat's scalp, and the ground wire was connected to the shield. The data were collected using biological signal acquisition and processing software (Chengdu Travel Electronic Company Limited, Chengdu, China), and the parameters were set according to experimental requirements (acquisition frequency of 12 frame/points, wave wide 1 ms, stimulation strength of 100 Hz, stimulus intervals of 200 ms). The electronic stimulator embedded in the system was used to stimulate the first part of a single square wave, which the stimulating electrode applied to the perforant pathway through the embedded stimulus isolator. The induced potential was input into the computer through an embedded bioelectric amplifier and automatically recorded as a storage wave. PS amplitude was then recorded. These data were considered as an index of dentate gyrus granulocyte excitability. When the experiment began, the stimulating electrode was first moved to stimulate the dentate gyrus and produce PS with a fixed stimulus. After the PS recording, the recording electrode was moved until the largest and longest-lasting PS was recorded[23].

Measurement methods of PS amplitude

Relative amplitude (%) of PS was considered as an observational index, and amplitude increases were observed. PS amplitude was recorded for 30 minutes before and after stimulus trains. Amplitude was measured at 6 different time points, and each time point was measured 6 times. PS amplitude at each time point was the average of 6 PS amplitudes. First, the average PS amplitude was calculated before the stimulus train. The average PS amplitude was considered as 100%. The PS amplitude at different time points was then divided by the average PS amplitude before the stimulus train at different time points. The relative amplitude (%) was then calculated at different time points (Figure 2)[24-25].

Statistical analysis

Experiment data were expressed as mean ± SD. SPSS 13.0 statistics software (SPSS, Chicago, IL, USA) was used to conduct tests of normality and homogeneity of variance. Data from the normal distribution and homogeneity tests were compared using one-way analysis of variance, and the *q* test was used for pair-wise comparison. Other data samples were compared using the H-rank test and the *q* test. A value of $P < 0.05$ was considered statistically significant.

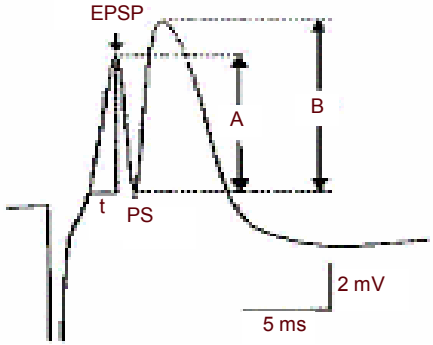

Figure 2 Population spike (PS) amplitude equals half of the addition of A and B.

EPSP: Excitatory postsynaptic potential; PS = (A+B)/2.

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Author contributions: Hongjiao Wan was responsible for the study concept. Changchun Cai designed the study. Xiaojin Yang analyzed experimental data. Cuiping Yang drafted the manuscript. Heping Ye was responsible for supervising the study. Yanping Yang, Zhigang Zhou and Jianhua Liu participated in the data acquisition and integration. Hongjiao Wan and Cuiping Yang organized the funding. Zhigang Zhou corrected the manuscript.

Conflicts of interest: None declared.

Ethical approval: The experiments were in accordance with the Animal Ethics Requirements set by the State and approved by the Ethics Committee of Jiangxi University of Traditional Chinese Medicine, China.

Supplementary information: Supplementary data associated with this article can be found, in the online version, by visiting www.nrronline.org, and entering Vol. 7, No. 15, 2012 after selecting the "NRR Current Issue" button on the page.

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